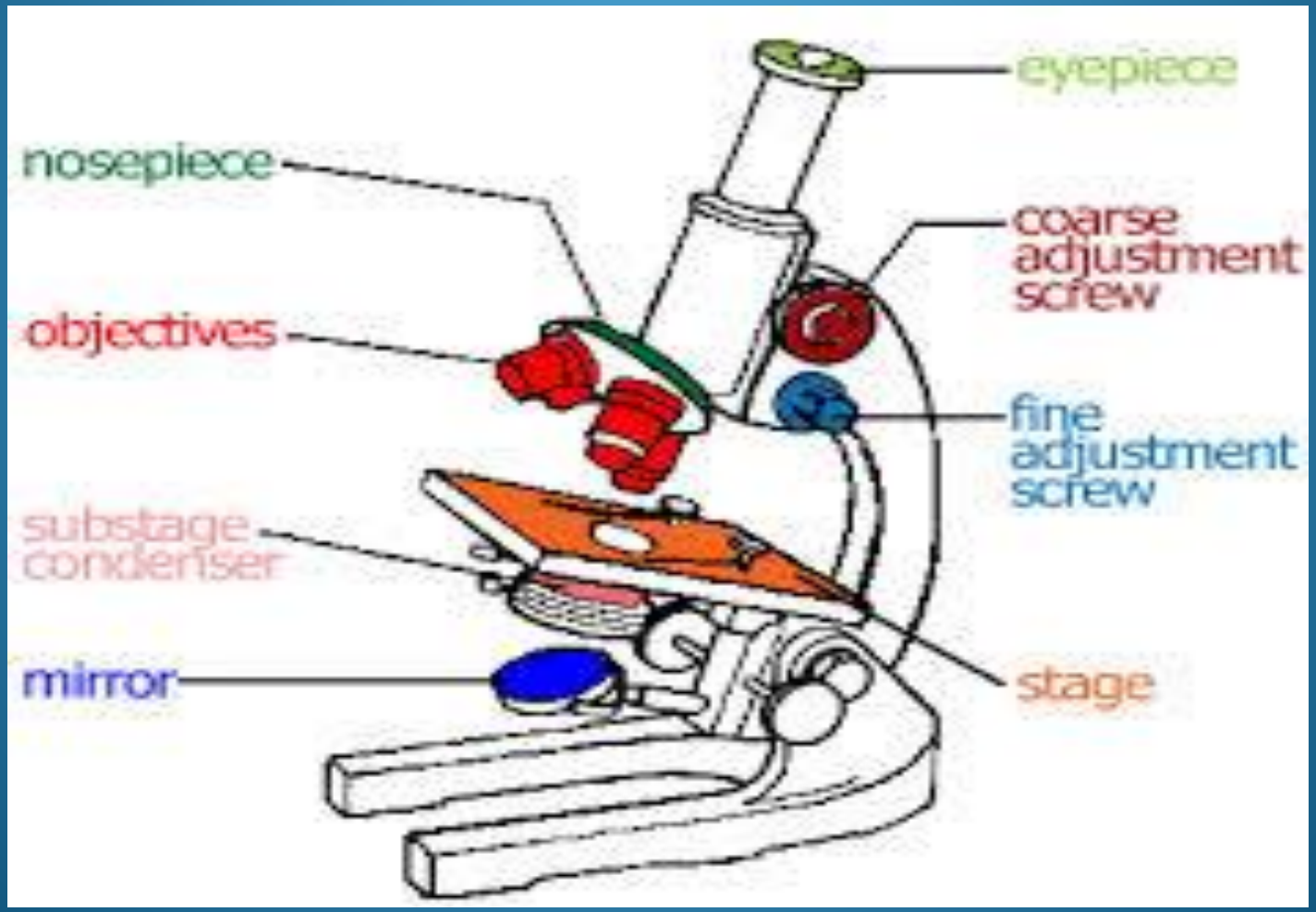
Microscopy & Micrometry



Microbiology

- Study of micro organisms.

Microscopy

- Technology of making very small things visible to human eye.

Resolving power

- It measures the ability to distinguish small objects close togather as separate and distinct objects.
- RP is minimum distance between 2 objects that reveal them as separate entities.

Optical instrument	Resolving power
Human eye	0.2 mm(200 µm)
Light microscope	0.2µm (200 nm)
Electron microscope	0.2 to 0.5 nm

Types

- Based on principles of magnification
  - Light or optical microscope
  - Electron microscope

Light Microscopes

most useful microscope available to the microbiologist.

- **Advantages:**

❖convenient

❖relatively less expensive

❖ easy to use

❖widely available.

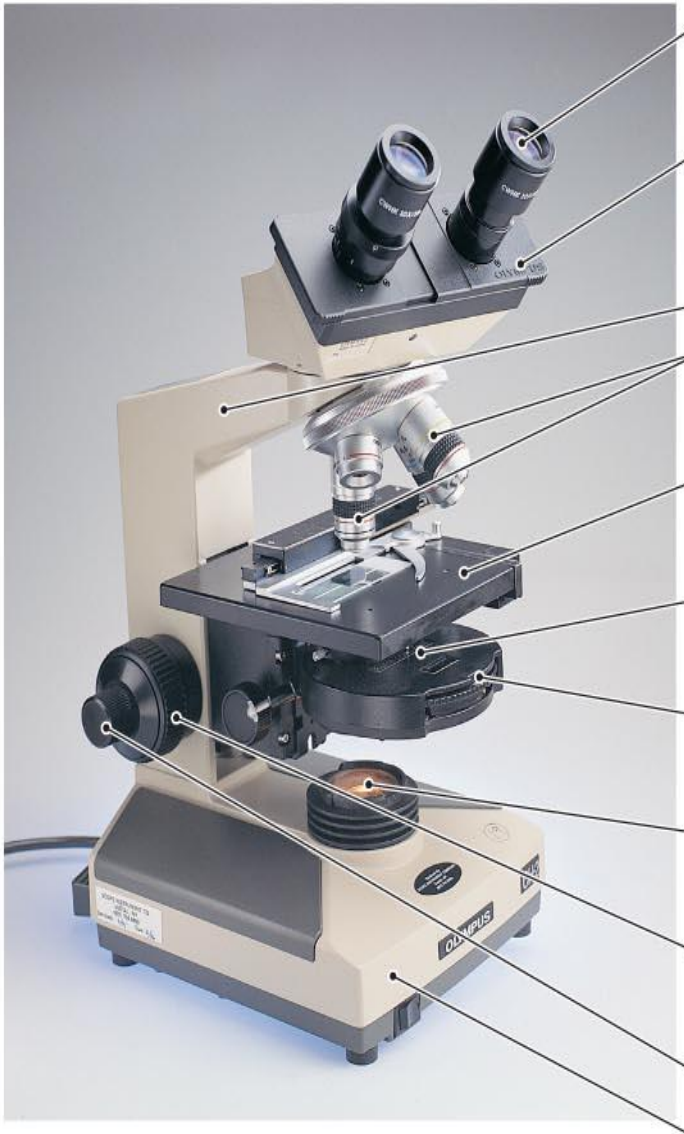
- **Disadvantages:**
- resolving power 0.2 micrometers at best (can recognize cells but cannot visualized its detailed internal structures).

Types

- Bright field(Compound light microscope)
- Phase contrast
- dark field
- Fluorescent

1)The Bright-Field Microscope

- It is most routinely used microscope.
  - Cells contain mainly water and do not contrast with the medium.
  - It needs contrast, so easiest way to view cell is to fix and stain.
  - **Principal:** Light passes through specimen into objective lens produces a dark image against a brighter background.
- Total magnification: It is product of the magnifications of the ocular lens and the objective lens



Ocular lens
Remagnifies the image formed by the objective lens

Body
Transmits the image from the objective lens to the ocular lens using prisms

Arm

Objective lenses
Primary lenses that magnify the specimen

Stage
Holds the microscope slide in position

Condenser
Focuses light through specimen

Diaphragm
Controls the amount of light entering the condenser

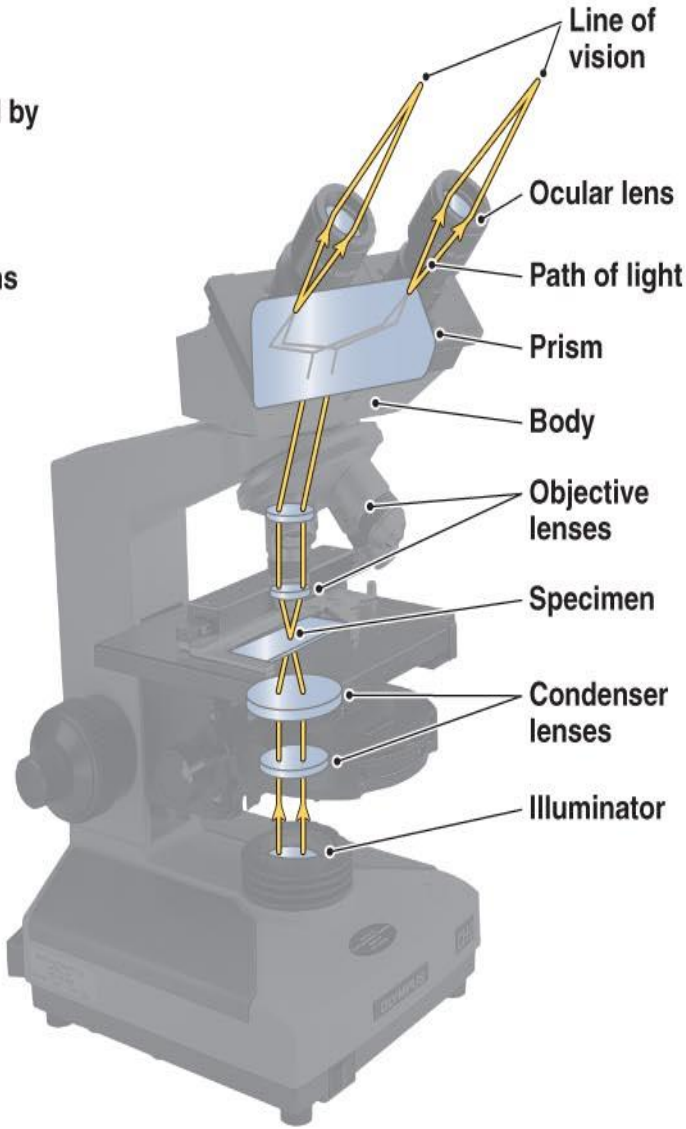
Illuminator
Light source

Coarse focusing knob
Moves the stage up and down to focus the image

Fine focusing knob

Base

(a)



(b)

Magnification = Power of Objective lens ✖ Power of ocular lens

Ocular Lens

- The ocular lens has a 10X magnification.

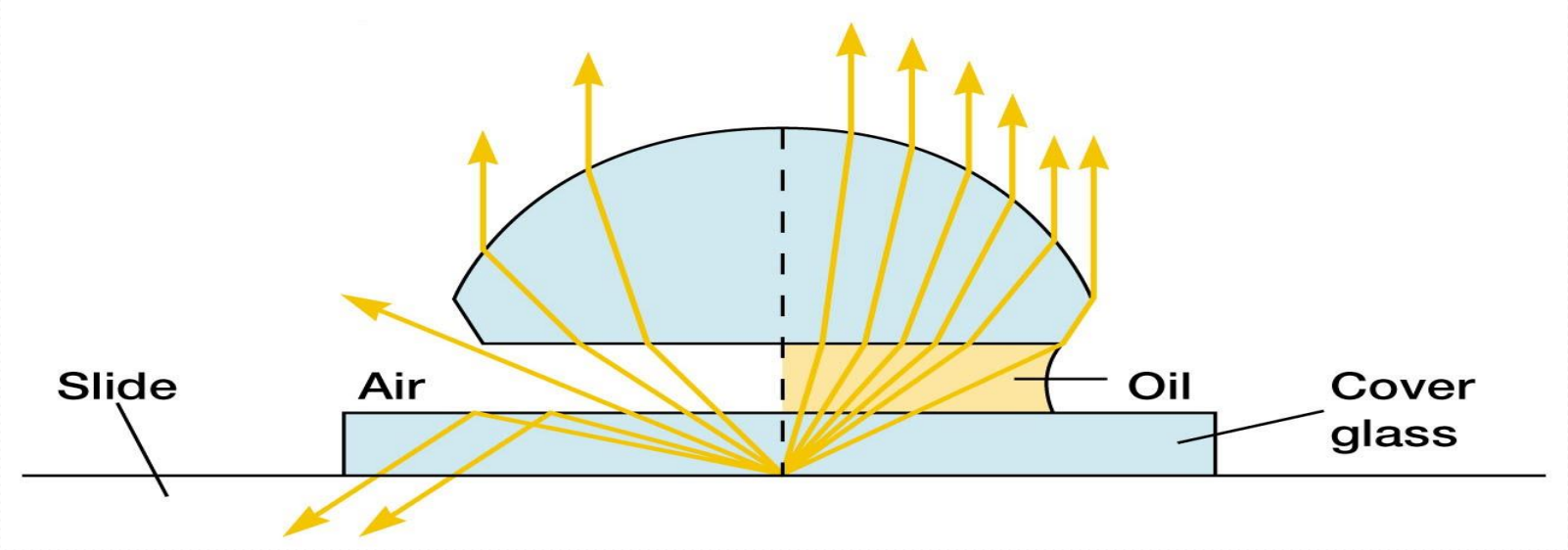
Objective Lens

- There are four objective lenses.
- Scanner – 4X
- Low power – 10X
- High power – 40X
- Oil immersion – 100X

- The ability of a microscope objective lens to capture deviated light rays from a specimen is dependent upon both the numerical aperture and the medium (e.g. Immersion oil) through which the light travels.

- Immersion oil is mainly used while examination under 100 X objectives.

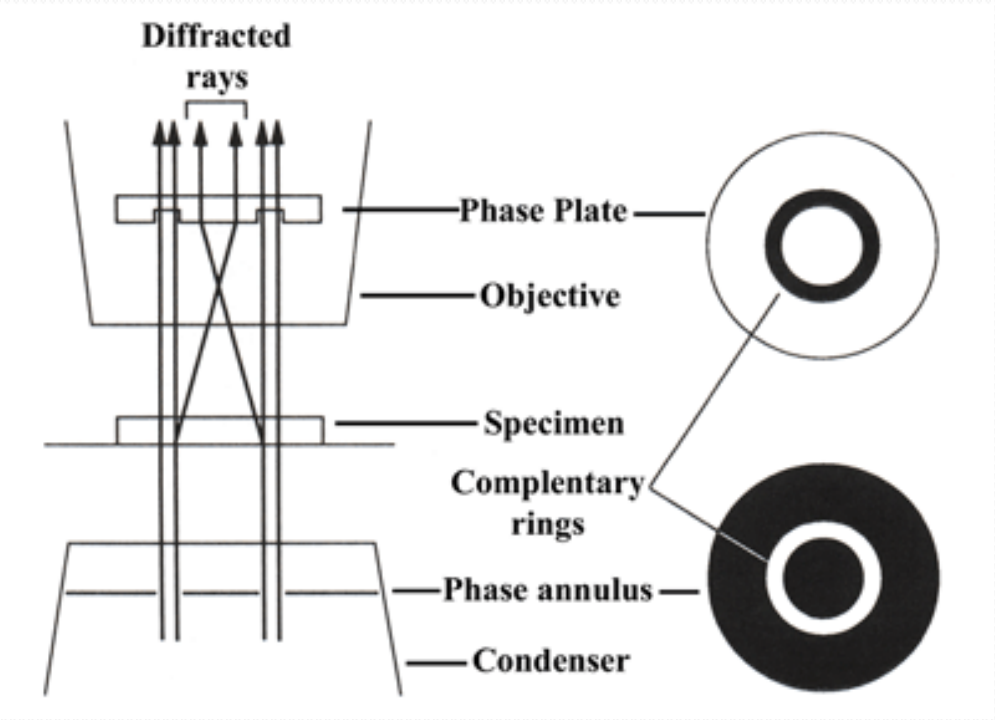
- . While passing of light from object (slide) to object lens (100 X) the light rays will be deviated due air in between these two and will make the vision blurred.
- The refractory index of immersion oil is equal to that of the glass The immersion oil replace the air in between these two glass material thus, prevent the deviation of light and make the vision more clear.
- cedarwood oil is most commonly used.



2)The Phase-Contrast Microscope

- enhances the contrast between intracellular structures having slight differences in refractive index
- excellent way to observe living cells
- When rays of light are passed through an object, they emerge in different phases depending on the difference in the refractive indices between the object & the surrounding medium & these phase differences are converted into differences in intensity of light producing light and dark contrast in the image.

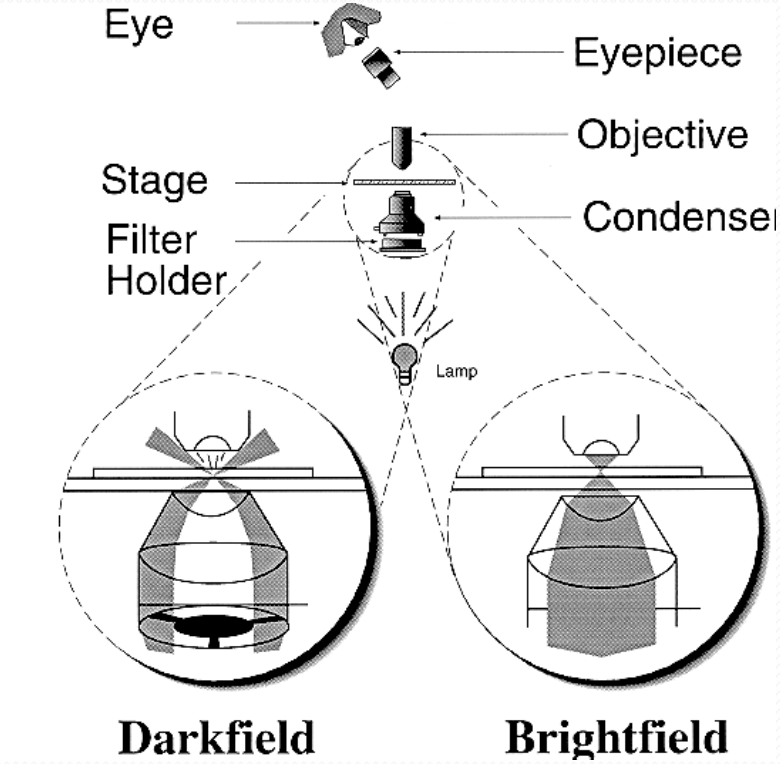
- A special condenser with an annular stop and a special phase objective with a phase plate are required.
  Uses: For the detection of bacterial components such as endospores & inclusion bodies. Widely used in studying eukaryotic cells



3) Dark Field Microscope:

- Principle: A special type of dark field condenser with a central circular stop is used, which illuminates the object with a cone of light without allowing light rays to enter directly the objective lens and only light rays reflected or scattered from the object enter the objective lens with the result that the object appears self-luminous against a dark background.
  - Best for observing pale objects
  - Only light rays scattered by specimen enter objective lens
  - Specimen appears bright against dark background
  - Increases contrast and enables observation of more details

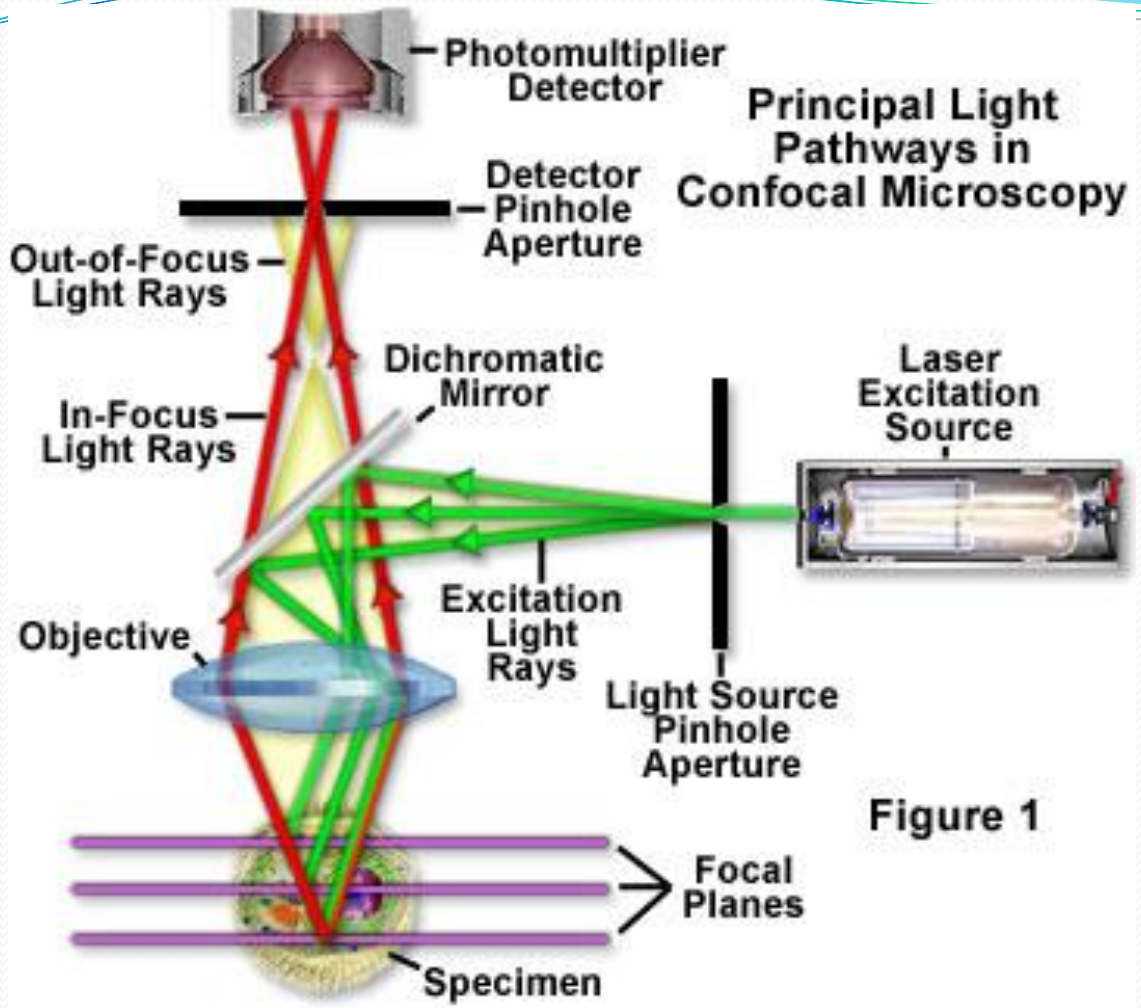
An ordinary microscope can be converted into dark field microscope by using a special type of dark-field condenser with a central circular stop, a funnel stop. Dark field microscope will improve the resolution power.



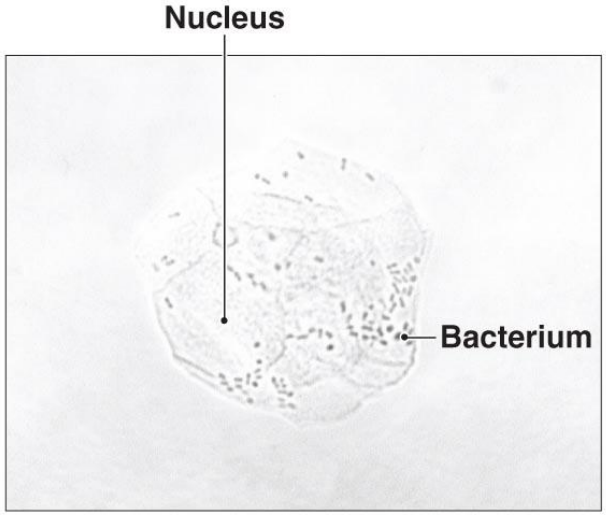
- Uses: -To demonstrate extremely slender organisms like spirochetes -To demonstrate motility -To demonstrate microfilaria in blood

4) Fluorescent Microscope:

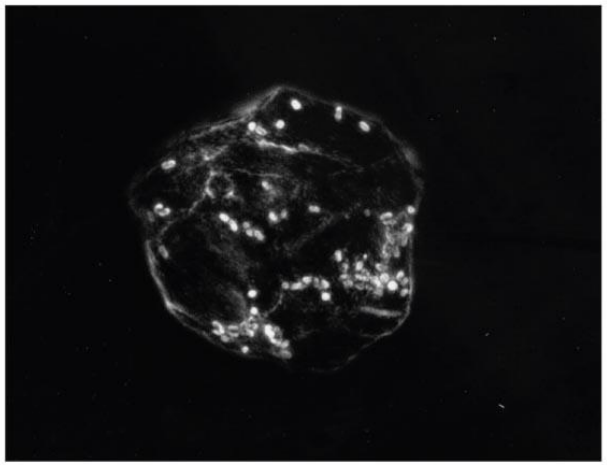
- **Principle:** Fluorescence is the property of absorbing light rays of one wavelength and emitting light rays of another wave length. The specimen is exposed to ultraviolet (UV) light of shorter wavelength that results in emission of longer wave length of visible light and organisms stained with fluorescent dyes become self-luminous and are seen as bright objects against a dark background.
- Fluroscent dyes commonly used are - Auramine rhodamine, Acridine orange, Calcofluor white.


Photomultiplier Detector
Principal Light Pathways in Confocal Microscopy
Detector Pinhole Aperture
Out-of-Focus Light Rays
Dichromatic Mirror
Laser Excitation Source
In-Focus Light Rays
Excitation Light Rays
Objective
Light Source Pinhole Aperture
Figure 1
Focal Planes
Specimen

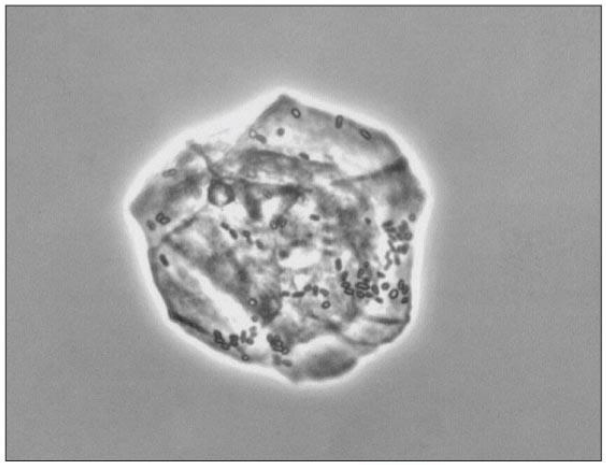
- **Uses: -**To visualize organisms like Mycobacterium tuberculosis, Trichomonas vaginalis, Entameoba histolytica cysts, malarial parasites and intracellular Gonococci and Meningococci as well as malarial parasites.
- -To detect antigen in tissue or specimen
- -To detect antibodies in serum.



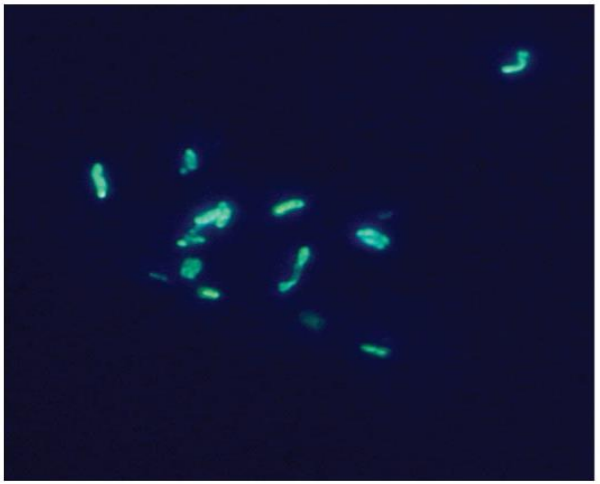
(a) Bright field LM 20 μm



(b) Dark field LM 20 μm



(c) Phase contrast LM 20 μm

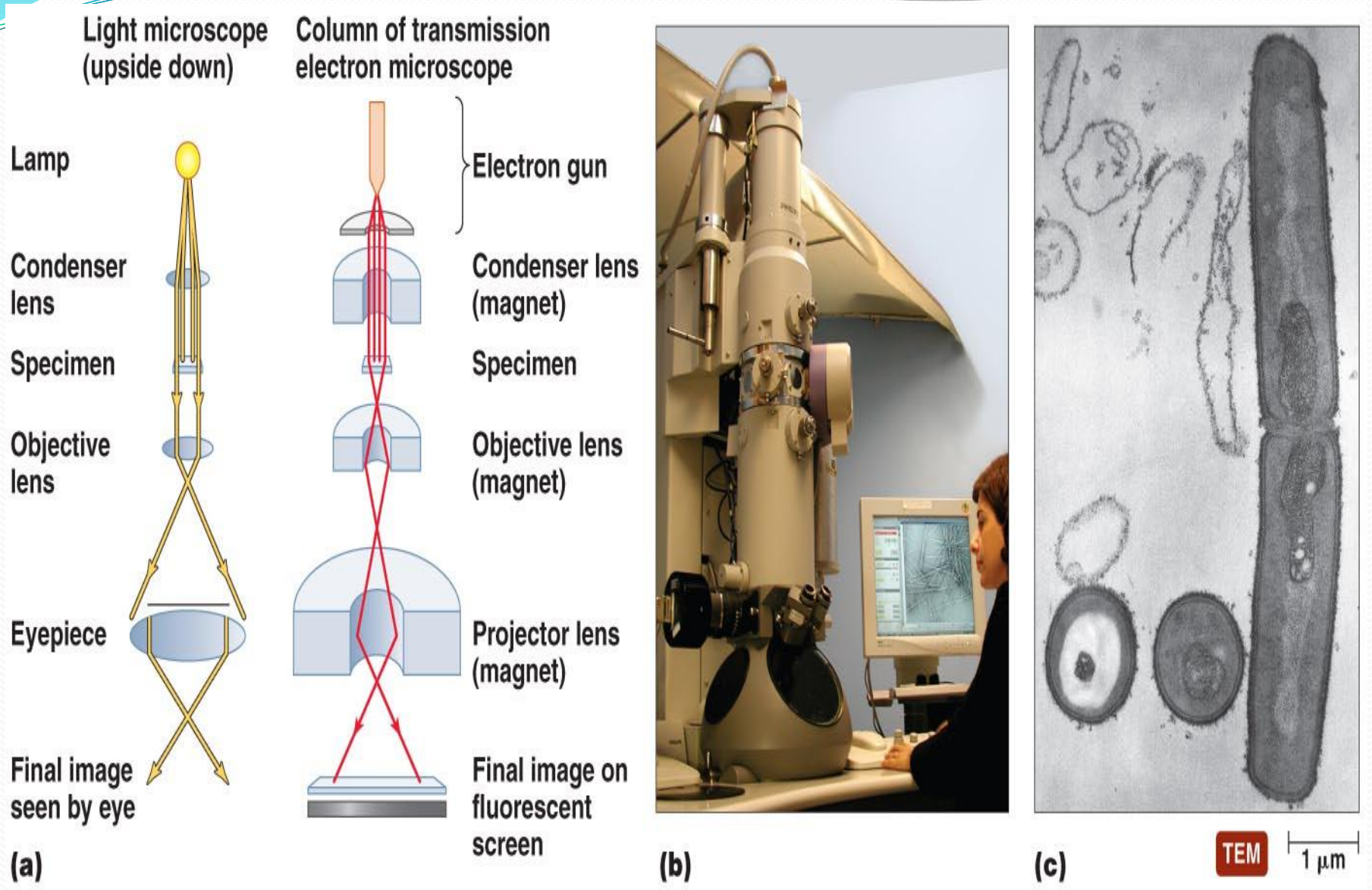


(b) LM 10 μm

Electron Microscope

- **Principle:** In this microscope, instead of light, a beam of electrons is used. The wavelength of electron is approximately 0.005 nm, as compared to 500 nm of visible light. The resolving power of electron microscope is 1,00,000 times that of light microscope.
- Two types of electron microscopes are available
  - Transmission electron microscope
  - Scanning electron microscope
- **Uses:** -To study various fine structures of bacteria & viruses.

  -To visualize very small organisms such as viruses.


Light microscope (upside down)
Column of transmission electron microscope
Lamp
Condenser lens
Specimen
Objective lens
Eyepiece
Final image seen by eye
Electron gun
Condenser lens (magnet)
Specimen
Objective lens (magnet)
Projector lens (magnet)
Final image on fluorescent screen
(a)
(b)
(c)
TEM
1 µm

Micrometry

❖The measurement of objects under the microscope using a calibrated eyepiece scale (micrometer) is called micrometry.

❖There are two parts of micrometer.

❑Eye piece or Ocular micrometer - consisting of uniformly spaced lines of unknown distance ranging from 0- 100 .

❑Stage micrometer – consisting of uniformly spaced lines of known distance of 0.01 mm b/w two divisions.

Method

- Place ocular and stage micrometer at their respective sites and adjust ocular lens in such way so that lines on left end of both micrometers match.

- Now calculate actual distance b/w lines of ocular micrometer by observing how many lines of ocular micrometer match with stage micrometer.
- this value is specific for particular stage- ocular micrometer combination only and very with different sets.
- . Now remove stage micrometer and palce the slide to measure size of any structure.

Thank You